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Assessment of bacterial infection in chronic wounds in the elderly: Biopsy versus VERSAJET

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ABSTRACT

Aim: The aim of this study was to evaluate the hydro-surgery VERSAJET system as a suitable alternative to the traditional invasive tissue sampling technique in detecting bacteria and their load in chronic wounds in the elderly. **Materials and methods**: To investigate and evaluate bacterial incidence and load in chronic wounds, we simultaneously performed on 19 affected patients a deep tissue biopsy and tissue collections by the VERSAJET hydro-surgical system. After local cleaning and anesthesia, a deep biopsy was performed with a punch of 3–4 mm in diameter. Subsequently, three tissue samples were collected by the VERSAJET system: one from the first washing in order to investigate the superficial contamination; one from the second washing to investigate deep tissue infection investigation and one from the third washing as a control procedure. After treatment, all tissue samples were cultured in vitro for diagnostic and micro-biological assessment. **Results**: Nineteen patients with chronic wounds of the lower limbs were enrolled from February 2010 to May 2013. Concordance between deep tissue biopsy cultures and tissue cultures collected by the VERSAJET system was examined. The deep tissue biopsy cultures showed complete concordance with the VERSAJET as follows: 2 patients (11%) for the first washing sample; 10 patients (53%) for the second washing sample; 4 patients (21%) for the third washing sample. However, with reference to only aerobic isolated strains, the concordance of the VERSAJET second washing samples cultures with a biopsy of the deep tissue cultures was very high (84%) and fairly high (63%) in the anaerobic isolated strains. The second VERSAJET washing sample cultures seem to have the highest concordance with the biopsy of the deep tissue cultures. **Conclusions**: Tissue biopsy remains the leading technique for detecting bacteria and their load in chronic wounds. However, this study shows that the hydro-surgery VERSAJET system is sufficiently effective in detecting bacteria and their load in chronic wounds and can be a potential alternative to a biopsy. In particular, the second washing sample culture showed the best correlation with the deep tissue biopsy culture. However, further studies are needed in order to modify techniques of tissue collection in the VERSAJET system before drawing any conclusions.

1. Introduction

Aerobic and anaerobic bacteria play an active and critical role in the chronic wound pathogenesis [1]. Frequently, lesion progression towards chronicity is related to an increase in affected tissue of bacteria load [2]. As the bacterial load increases so will the wound healing time. The debridement is essential for the wounds healing and several techniques are available to remove the necrotic tissue and reduce bacterial load to convert a chronic into an acute wound.

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The surgical debridement of the necrotic tissue is the gold standard and is needed for treating the wound; however, this practice, even if carried out quickly, can be painful and non selective because it can remove healthy tissue. A variety of mechanical methods for wound debridement are being evaluated and one of the most promising is the hydro-surgery system (VERSAJET) [3].

The VERSAJET is a debridement tool based on the Venturi effect which simultaneously produces demolition and removal of the necrotic tissue and drastically reduces bacterial load [5]. This technology uses a sterile water jet at variable power settings [4,5] and is better tolerated by patients. The deep tissue sampling by biopsy is an invasive technique, which requires technical skills and is unpleasant to patients [6]. Therefore, evaluation of the VERSAJET usefulness in performing specimens for both diagnostic and microbiological assessment appeared very interesting.

The aim of this study was to evaluate the VERSAJET usefulness in detecting bacteria and their load in chronic wounds in the elderly.

2. Materials and methods

2.1. Our population

Nineteen patients affected by chronic wounds on the lower limbs with evident infection supported by laboratory findings (presence of necrotic tissue, purulent exudates, neutrophilic leukocytosis, etc.) afferent to the Medical Angiology Department of the Second University of Naples were enrolled.

All patients signed the form for informed consent.

2.2. Tissue sampling

To investigate and evaluate the bacterial load, before any antibiotic therapy or ten days after antibiotic withdrawal, we simultaneously performed on patients, both a biopsy of the deep tissue and three tissue samples by hydro-surgical VERSAJET system.

The deep tissue biopsy was performed with a punch of 3–4 mm in diameter after local anesthesia and cleaning. The specimen was placed in a sterile container with screw closure, without fixation [7,8].

The VERSAJET sampling was performed after wound surface cleansing with power set at 3 as follows:

- A primary collection of 100 ml of liquid (first washing on the whole ulcer area with power set to 5) to investigate on superficial contamination.
- A second collection of 250 ml (second washing on the edges and at the center of the lesion with power set at 7 or more) to investigate on deep tissue infections.
- A third collection (third washing, as the second one, but set at the maximum tolerated power of 9 or 10) as control procedure control and standardized support.

All three samples were collected in sterile containers and sent, together with the biopsy tissue, to the Microbiological Department of the Second University of Naples.

The time lapse from specimens sampling and processing never exceeded 20 min.

2.3. Specimens processing

All steps were performed under laminar flow hood.

2.3.1. Deep tissue

Two grams of deep tissue were aseptically transferred in a 15 ml test tube and ground with a sterile tissue-milling machine (Griffiths test tube) and then homogenized adding 1 ml of sterile Brain Hearth Infusion broth. Homogenate was diluted with sterile Brain Heart Infusion broth to the final volume of 5 ml and again homogenized by the vortex for 10–15 s.

Table 1
Basal characteristics of patients.

Characteristics of patients	Venous ulcer ($n = 8$)	Arterial ulcer ($n = 6$)	Ulcer mixed ($n = 5$)	Total ($n = 19$)
Gender, no. (%)				
Male	3 (38%)	2 (33%)	1 (20%)	**6 (31.6%)**
Female	5 (62%)	4 (67%)	4 (80%)	**13 (68.4%)**
Age (years)				
Median	68	70	68	**68.5**
Range	*(65–71)*	*(67–73)*	*(67–69)*	***(65–73)***
Smokers, no. (%)				
Yes	5 (62%)	4 (67%)	5 (100%)	**14 (73.7%)**
No	3 (38%)	2 (33%)	–	**5 (26.3%)**
Comorbidities, no. (%)				
Diabetes mellitus type 2	1 (12.5%)	–	–	**1 (5.2%)**
Hypertension	2 (25%)	6 (100%)	2 (100%)	**10 (52.6%)**
Ischemic heart	2 (25%)		1 (20%)	**3 (15.8%)**
Peripheral vascular disease	1 (12.5%)	6 (100%)	2 (40%)	**9 (47.4%)**
Degenerative arthropathy	2 (25%)	2 (33%)	1 (20%)	**5 (26.3%)**
Metabolic disorders	8 (100%)	4 (67%)	3 (60%)	**15 (78.9%)**
Other	3 (38%)	2 (33%)	2 (33%)	**7 (36.8%)**

2.3.2. VERSAJET samples

Each washing liquid specimen was shaken by the vortex for 10–15 s and vacuum filtered through a 0.22 μm membrane (Millipore® Stericup and Steritop). The filtered liquid was eliminated; 2 g of the material deposited on the membrane surface was transferred in a 15 ml test tube and diluted with sterile Brain Heart Infusion broth to a final volume of 5 ml and homogenized by the vortex for 10–15 s.

2.4. Microbiological assessment

100 μl of each sample were seeded on the following media:

Chocolate Polivitex (PVX) agar, Columbia CNA agar with 5% of sheep blood (CNA), Mac-Conkey agar (MCK), Sabouraud agar (SAB), Mannitol Salt agar (MAN).

The CNA, MCK and MAN agars plates were incubated in aerobiosis at 37 °C for 24 h; the SAB Agar plates were incubated in aerobiosis at 30 °C for 24–48 h; one PVX agar plate was incubated in microaerophilia at 37 °C for 24–48 h and an additional PVX agar plate was incubated in anaerobiosis at 37 °C for 48–72 h.

2.4.1. Bacterial identification

The bacterial strains developed in aerobiosis or in microaerophilia were identified by GN1341 for Enterobacteriaceae and GP21342 for Gram-positive microorganisms. Reading of biochemical tests was obtained with the automatic reader (Vitek® 2 system Biomerieux).

The bacterial strains developed in anaerobiosis were identified by RAPID ID 32 A test (Biomerieux®), a standardized system with 29 miniaturized enzymatic tests. The test reading was carried out with (ATB® Expression®) software 4 h after anaerobiosis incubation at 37 °C.

Yeast was identified by ID 32 C test (Biomerieux®), a standardized system containing 32 miniaturized assimilation tests. The test

reading was carried out with an identification software (ATB® Expression®) 24–48 h after aerobiosis incubation at 30 °C.

Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Capnocytophaga sputigena* ATCC 33612, *Bacteroides fragilis* ATCC 23745 and *Candida glabrata* ATCC 64677 were used as the reference strains.

3. Results

3.1. Patients clinical characteristics

Nineteen patients (6 males (31.6%) and 13 females (68.4%)) with a mean age of 68.5 years (range 65–73) were enrolled (Table 1). Eight patients were suffering from venous ulcer, 6 from arterial ulcer and 5 from mixed lesions. Fourteen patients (73.7%) smoked, ten (52.6%) were suffering from arterial hypertension, nine (47.4%) from peripheral vascular disease and fifteen (78.9%) from metabolic disorder.

3.2. Bacteriological results

A similar number of strains were isolated from cultures of both, biopsy removed deep tissue (4.15; range 2–6) and VERSAJET second washing collected tissue (4.1; range 2–6); while, a mean of 2.15 strains (range 1–4) were isolated from VERSAJET first washing collected tissue and a mean of 3.6 strains (range 0–6) from the VERSAJET third washing collected tissue (Table 2).

The number of aerobic isolated strains from different samples showed a mild difference: from 1.89 (third washing) to 2.4 (second washing), while the number of the anaerobic isolated strains was quite different: from 0.26 (first washing) to 1.89 (deep tissue). The deep tissue biopsy cultures showed a similar number of aerobic and anaerobic strains (2.26 and 1.89, respectively: range 0–4); while the second VERSAJET washing collected tissue cultures showed more aerobic than anaerobic strains [2.4 (range 1–4) and 1.7 (range 0–4), respectively]. A more striking difference between the aerobic and anaerobic isolated strains resulted in the first [1.89 (range 0–4) and 0.26 (range 0–1)] and third [2.0 (range 0–4) and 1.58 (range 0–3)] VERSAJET washing samples.

Tables 3 and 4 show aerobic and anaerobic isolated bacteria in each patient. Patient n. 9 affected by venous ulcer showed no aerobic bacteria growth, but only anaerobic bacteria growth, while in two patients (n. 6 and n. 19) with only arterial ulcer, aerobic bacteria growth was observed. Fungi were isolated from 4 specimens; in patient n. 14 *Candida albicans* was isolated from second and third washing samples, but not from the first sample nor from the deep tissue. In patient n. 18 *Candida tropicalis* was isolated from the deep tissue and the second and third washing samples but not from the first washing sample. *Candida parapsilosis* was isolated in 2 patients: in patient n. 6 from all specimens and in patient n. 1 only from deep tissue and from second VERSAJET washing specimen.

Tables 5 and 6 show the aerobic and anaerobic isolated bacteria in the 19 patients. Forty-three aerobic bacteria and 36 anaerobic bacteria were isolated from deep tissue samples. Thirty-six aerobic and 7 anaerobic bacteria were isolated from the first VERSAJET washing samples; 45 aerobic and 33 anaerobic bacteria from the second washing samples and 37 aerobic and 30 anaerobic bacteria from the third washing samples.

Table 2
Number of bacterial strains isolated.

Type of specimen	No. of patients	No. of strains per specimen		
		Aerobes	Anaerobes	Total
Deep tissue	19	2.26	1.89	**4.15**
First washing	19	1.89	0.26	**2.15**
Second washing	19	2.4	1.7	**4.1**
Third washing	19	2.0	1.58	**3.58**

Table 3
Aerobic microorganisms isolated.

Patient no.	Microorganisms isolated by			
	Deep tissue	First washing	Second washing	Third washing
1	*S. haemolyticus* *P. aeruginosa* *C. striatum* *Candida parapsilosis*	*S. haemolyticus* *P. aeruginosa* *C. striatum* –	*S. haemolyticus* *P. aeruginosa* *C. striatum* *Candida parapsilosis*	*S. haemolyticus* *P. aeruginosa* – –
2	*Enterococcus faecalis* *C. striatum* *Enterobacter cloacae* *Klebsiella oxytoca*	*Enterococcus faecalis* *C. striatum* *Enterobacter cloacae* *Klebsiella oxytoca*	*Enterococcus faecalis* *C. striatum* *Enterobacter cloacae* *Klebsiella oxytoca*	*Enterococcus faecalis* *C. striatum* *Enterobacter cloacae* *Klebsiella oxytoca*
3	*S. equisimilis* *E. faecalis* – –	– – *S. simulans* *Corinebacterium* spp	– – *S. simulans* *Corinebacterium* spp	– – *S. simulans* *Corinebacterium* spp
4	*Brevibcaterium* spp *P. aeruginosa* *Citrobacter freundii*	*Brevibcaterium* spp *P. aeruginosa* *Citrobacter freundii*	*Brevibcaterium* spp *P. aeruginosa* *Citrobacter freundii*	*Brevibcaterium* spp *P. aeruginosa* *Citrobacter freundii*
5	*S. haemolyticus* *S. agalactiae* – –	*S. haemolyticus* – *P. putida* –	*S. haemolyticus* – *P. putida* *C. striatum*	*S. haemolyticus* – *P. putida* *C. striatum*
6	*S. simulans* *Candida parapsilosis*	*S. simulans* *Candida parapsilosis*	*S. simulans* *Candida parapsilosis*	*S. simulans* *Candida parapsilosis*
7	*S. aureus* *P. aeruginosa* *M. morganii*	*S. aureus* *P. aeruginosa* *M. morganii*	*S. aureus* *P. aeruginosa* *M. morganii*	*S. aureus* *P. aeruginosa* *M. morganii*
8	*S. aureus* *E. coli* *E. faecalis* *E. avium*	*S. aureus* *E. coli* – –	*S. aureus* *E. coli* *E. faecalis* *E. avium*	*S. aureus* *E. coli* *E. faecalis* –
9	None	None	None	None
10	*S. haemolyticus* *S. marcescens*	*S. haemolyticus* *S. marcescens*	*S. haemolyticus* *S. marcescens*	*S. haemolyticus* *S. marcescens*
11	*S. aureus*	*S. aureus*	*S. aureus*	*S. aureus*
12	*S. aureus* *P. aeruginosa*	*S. aureus* *P. aeruginosa*	*S. aureus* *P. aeruginosa*	*S. aureus* *P. aeruginosa*
13	*S. epidermidis* *E. faecalis* *P. rettgeri*	*S. epidermidis* *E. faecalis* –	*S. epidermidis* *E. faecalis* *P. rettgeri*	– *E. faecalis* *P. rettgeri*
14	*E. coli* *M. morganii* –	*E. coli* *M. morganii*	*E. coli* *M. morganii* *C. albicans*	*E. coli* *M. morganii* *C. albicans*
15	*E. faecalis* *S. malthophilia*	*E. faecalis* –	*E. faecalis* *S. malthophilia*	– *S. malthophilia*
16	*S. aureus* *P. mirabilis*	*S. aureus* *P. mirabilis*	*S. aureus* *P. mirabilis*	*S. aureus* *P. mirabilis*
17	*S. equisimilis* *P. aeruginosa*	– *P. aeruginosa*	– *P. aeruginosa* *Citrobacter freundii*	– *P. aeruginosa* *Citrobacter freundii*
18	*Candida tropicalis*	–	*Candida tropicalis* *Brevibacterium* spp	*Candida tropicalis* –
19	*S. epidermidis* *Corinebacterium* spp	*S. epidermidis* *Corinebacterium* spp	*S. epidermidis* *Corinebacterium* spp	– –

Table 4
Anaerobic microorganisms isolated.

Patient no.	Microorganisms isolated by:			
	Deep tissue	First washing	Second washing	Third washing
1	*P. acnes*	–	*P. acnes*	–
	F. nucleatum	–	*F. nucleatum*	*F. nucleatum*
2	*B. fragilis*	–	*B. fragilis*	*B. fragilis*
	P. anaerobius	–	*P. anaerobius*	*P. anaerobius*
3	None	None	None	None
4	*B. fragilis*	*B. fragilis*	*B. fragilis*	*B. fragilis*
	P. magnus	–	*P. magnus*	*P. magnus*
			F. nucleatum	*F. nucleatum*
5	*P. asaccharolyticus*	–	*P. asaccharolyticus*	*P. asaccharolyticus*
	B. capillosus	–	–	*B. capillosus*
6	None	None	None	None
7	*Peptostreptococcus* spp	*Peptostreptococcus* spp	*Peptostreptococcus* spp	–
	C. perfringens	–	*C. perfringens*	*C. perfringens*
8	*P. prevotii*	–	*P. prevotii*	*P. prevotii*
	B. putredinis	–	–	*B. putredinis*
9	*Prevotella* spp	*Prevotella* spp	*Prevotella* spp	*Prevotella* spp
	C. sporogenes		*C. sporogenes*	*C. sporogenes*
	F. varium		*F. varium*	–
10	–	–	Unidentified coccus	Unidentified coccus
11	*P. asaccharolyticus*	–	*P. asaccharolyticus*	*P. asaccharolyticus*
	C. perfringens	–	–	–
12	*C. bifermentans*	–	*C. bifermentans*	–
	F. nucleatum		*F. nucleatum*	*F. nucleatum*
13	*B. melaninogenicus*	–	*B. melaninogenicus*	*B. melaninogenicus*
	P. anaerobius	–	*P. anaerobius*	*P. anaerobius*
14	*P. acnes*	*P. acnes*	*P. acnes*	–
	B. multiacidus	–	–	–
	C. sporogenes	–	*C. sporogenes*	*C. sporogenes*
15	*C. cadaveris*	–	*C. cadaveris*	*C. cadaveris*
	P. anaerobius	*P. anaerobius*	*P. anaerobius*	*P. anaerobius*
	F. mortiferum	–	*F. mortiferum*	*F. mortiferum*
	Lactobacillus spp	–	–	–
16	*P. prevotii*	–	*P. prevotii*	*P. prevotii*
17	*C. perfrigens*	*C. perfrigens*	*C. perfrigens*	*C. perfrigens*
	F. nucleatum	–	*F. nucleatum*	*F. nucleatum*
	B. fragilis	–	*B. fragilis*	*B. fragilis*
			B. melaninogenicus	*B. melaninogenicus*
18	*P. asaccharolyticus*	*P. asaccharolyticus*	*P. asaccharolyticus*	*P. asaccharolyticus*
	P. acnes	–	*P. acnes*	*P. acnes*
	B. multiacidus	–	*B. multiacidus*	*B. multiacidus*
	C. bifermentans	–	–	–
19	None	None	None	None

S. aureus and *E. faecalis* resulted as the most frequently isolated Gram-positive bacteria while *P. aeruginosa* was the most frequently isolated Gram-negative bacteria (Table 5).

Peptostreptococcus anaerobius, *Clostridium perfrigens*, *B. fragilis* and *Fusobacterium nucleatum* were the most frequently isolated anaerobic bacteria (Table 6).

Fig. 1 reported the bacterial growth density per gram of tissue. Regardless of the tissue sampling techniques, bacterial load resulted low in all specimens; in deep tissue biopsy, mean bacterial growth density × 1000/g of tissue was 350 CFU (range 10–1000); however, the anaerobic bacteria growth density resulted higher than aerobic bacteria (mean × 1000/g of tissue: 550 CFU vs. 220 CFU, respectively). Similarly low bacterial growth density resulted in the first, second and third VERSAJET washing samples [mean × 1000/g of tissue = 200 CFU (range 5–1000), 330 CFU (range 20–1000) and 80 CFU (range 2–750), respectively]. Furthermore, the anaerobic bacteria growth density resulted quite differently from aerobic growth density in the first washing VERSAJET sample [mean × 1000/g of tissue = 350 CFU (range 5–1000) for aerobic bacteria vs. 80 CFU (range 2–200) for anaerobic bacteria], in the second washing sample [mean × 1000/g of tissue = 200 CFU (range 10–1000) for aerobic bacteria vs. 450 CFU (range 50–1000) for anaerobic bacteria] and in the third washing sample [mean × 1000/g of tissue = 50 CFU (range 2–400) for aerobic bacteria vs. 300 CFU (range 40–750) for anaerobic bacteria].

Table 7 compares the bacteriological results of the deep tissue biopsy cultures with the VERSAJET washing collected samples cultures. The results of the second VERSAJET washing samples cultures seem to have a better concordance with the results of the deep tissue biopsy cultures (10/19 (53%)), while the cultures concordant with deep tissue biopsy cultures were very low for the first (2/19 (11%)) and the third (4/19 (21%)) VERSAJET washing specimen. Moreover, considering the aerobic bacterial strains isolated in both, second VERSAJET washing samples and the deep tissue biopsy, the cultures concordance was very high (16/19 (84%)) and the cultures concordance for the anaerobic bacteria (12/19 (63%)) was high as well.

4. Discussion

The results of this study showed that hydro-surgery VERSAJET system is also effective in detecting microorganisms and their load in chronic wounds, in particular, the sampling showing the best concordance with the deep tissue biopsy cultures was the second washing collected sample.

Table 5
No. of aerobic microorganisms isolated.

Group or species	No. of isolated				
	Deep tissue	I Washing	II Washing	III Washing	Total
Aerobic	**43**	**36**	**45**	**37**	**161**
Gram-positive cocci	***20***	***15***	***17***	***13***	***65***
Staphylococcus aureus	5	5	5	5	20
Staphylococcus epidermidis	2	2	2	–	6
Staphylococcus haemolyticus	3	3	3	3	12
Staphylococcus simulans	1	2	2	2	7
Streptococcus equisimilis	2	–	–	–	2
Streptococcus agalactiae	1	–	–	–	1
Enterococcus faecalis	5	3	4	3	15
Enterococcus avium	1	–	1	–	2
Gram-positive bacilli	***4***	***6***	***7***	***3***	***20***
Corinebacterium spp	1	2	2	1	6
Corinebacterium striatum	2	2	3	1	8
Brevibacterium. Spp	1	1	1	1	4
Gram-negative bacilli	***16***	***16***	***20***	***20***	***72***
Pseudomonas aeruginosa	5	5	5	5	20
Pseudomonas putida	–	1	1	1	3
Stenotrophomonas malthophilia	1	–	1	1	3
Escherichia coli	2	2	2	2	8
Enterobacter aerogenes	–	–	–	–	–
Enterobacter cloacae	1	1	1	1	4
Citrobacter freundii	1	1	2	2	6
Klebsiella oxytoca	1	1	1	1	4
Proteus mirabilis	1	1	1	1	4
Providencia rettgeri	1	–	1	1	3
Morganella morganii	2	2	2	2	8
Serratia marcescens	1	1	1	1	4
Fungi	***3***	***1***	***4***	***3***	***11***
Candida parapsilosis	2	1	2	1	6
Candida tropicalis	1	–	1	1	3
Candida albicans	–	–	1	1	2

The concomitant concordance of both, aerobic and anaerobic isolated bacteria between deep tissue by biopsy sampling cultures and the VERSAJET second washing sampling cultures was observed in 10 patients (53%). However, only in the aerobic isolated strains

Table 6
No. of anaerobic microorganisms isolated.

Group or species	No. of isolated				
	Deep tissue	I Washing	II washing	III washing	Total
Anaerobic	**36**	**7**	**33**	**30**	**106**
Gram-positive cocci	***11***	***4***	***12***	***11***	***38***
Peptococcus magnus	1	–	1	1	3
P. asaccharolyticus	3	1	3	3	10
P. prevotii	3	1	3	3	10
Peptostreptococcus anaerobius	4	2	4	2	12
Unidentified coccus	–	–	1	1	2
Gram-positive bacilli	***12***	***2***	***10***	***8***	***32***
Clostridium bifermentans	2	–	1	–	3
C. perfrigens	3	1	2	2	8
C. sporogenes	2	–	2	2	6
C. cadaveris	1	–	1	1	3
Propionobacterium acnes	3	1	3	2	9
Lactobacillus spp	1	–	–	–	1
Gram-negative bacilli	***13***	***1***	***12***	***13***	***39***
Bacteroides fragilis	3	1	3	3	10
B. multiacidus	2	–	1	1	4
B. melaninogenicus	1	–	2	2	5
B. capillosus	1	–	–	1	2
B. putredinis	1	–	–	1	2
Fusobacterium nucleatum	3	–	4	4	11
F. varium	1	–	1	–	2
F. mortiferum	1	–	1	1	3

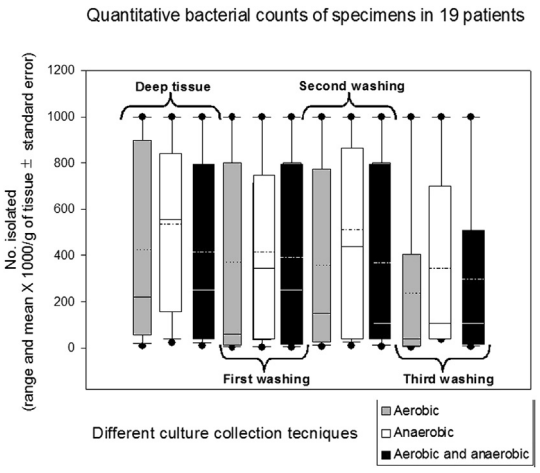

Fig. 1. Bacterial counts of specimens in 19 patients.

was the concordance of the VERSAJET second washing sample cultures with the deep tissue biopsy cultures very high (84%) and fairly high (63%) for the anaerobic isolated strains.

The deep tissue biopsy culture showed a higher sensitivity when compared with the second VERSAJET washing sample culture in detecting Gram-positive bacteria. In two patients affected by venous ulcers and in one patient affected by mixed ulcers, extremely invasive bacteria as *Streptococcus equisimilis* and *Streptococcus agalactiae* were isolated from deep tissue culture only and not from the second VERSAJET washing specimen cultures.

On the other hand, the second VERSAJET washing specimen culture showed better sensitivity when compared with the deep tissue biopsy culture in detecting Gram-negative bacteria. In two patients with arterial ulcer, *Enterobacter cloaca*, *Citrobacter freundii* and *Pseudomonas putrida* were isolated from the second VERSAJET washing sample and not from the biopsy of the deep tissue.

The different sensitivity shown by the deep tissue sampling biopsy and the VERSAJET washing sampling in detecting Gram-positive and Gram-negative aerobic and anaerobic bacteria is not clear. This could be due to different sampling techniques. Therefore, further studies investigating different methods for washing liquid filtration and/or tissue collection sampling are needed before drawing any conclusions.

As reported in previous papers, this study confirms the major role of *S. aureus*, *E. faecalis*, *P. aeruginosa*, Enterobacteriacae and anaerobic bacteria in chronic wound infections [9–21].

In conclusion, the hydro-surgery VERSAJET system is a promising technique for chronic wound debridement. Despite having demonstrated a little lower sensitivity when compared with the deep tissue biopsy cultures, thanks to the minimally invasive tissue sampling and better patients' compliance can be considered as a valid alternative to deep tissue biopsy cultures especially in patients with few or no clinical signs of infection or as first line to the microbiological investigation in chronic wounds. Furthermore, because the tissue collection sampling is performed during an

Table 7
Concordance[a] with deep tissue culture results.

Sampling modality	No. of specimens with positive concordance/no. done (%)		
	Aerobes	Anaerobes	Aerobes and anaerobes
First washing	11/19 (59)	3/19 (16)	2/19 (11)
Second washing	16/19 (84)	12/19 (63)	10/19 (53)
Third washing	11/19 (58)	10/19 (53)	4/19 (21)

[a] Concordance was defined as "complete qualitative agreement in the bacteriological results".

ordinary debridement session, the washing VERSAJET sampling can also be employed to monitor the effectiveness of an antibiotic therapy and the evolution of wound healing.

List of abbreviations

PVX	Chocolate Polivitex agar
CNA	Columbia CNA agar
MCK	Mac-Conkey agar
SAB	Sabouraud agar
MAN	Manitol salt agar
CFU	colony forming units
ATCC	American Type Culture Collection

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Conflicts of interest

No conflict of interests.

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